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Oviposition preference of adult females of *Lycaeides argyrognomon* (Bergsträsser) (Lepidoptera: Lycaenidae) for three food plants: *Indigofera pseudo-tinctoria*, Chinese-grown *Indigofera* sp. and *Vicia cracca*

Eri OZAKI[1), Keiko KODA[2)]* and Hiroshi NAKAMURA[1)]

[1)] Education and Research Center of Alpine Field Science, Faculty of Agriculture, Shinshu University, Minamiminowa, Nagano, 399-4598 Japan

[2)] Institute Mountain Science, Shinshu University, Minamiminowa, Nagano, 399-4598 Japan

Abstract To investigate the oviposition preference of adult females of *Lycaeides argyrognomon* for three food plants: *Indigofera pseudo-tinctoria* (JPN-*I*), Chinese-grown *Indigofera* sp. (CHA-*I*) and *Vicia cracca*, two kinds of experiments using the Richard method were carried out in 2011 at the laboratory of the Faculty of Agriculture of Shinshu University. When three plants were sequentially presented, all females laid eggs on JPN-*I* and 66.7% of females laid eggs on CHA-*I*. By contrast, no females laid eggs on *V. cracca*. The numbers of eggs laid on JPN-*I* and CHA-*I* were 372 (77.8%) and 106 (22.2%), respectively. When JPN-*I* and CHA-*I* were simultaneously presented to each female, all females laid eggs on CHA-*I*. The numbers of eggs laid on JPN-*I* and CHA-*I* were 456 (87.7%) and 64 (12.3%), respectively. Based on these results, we believe that females of *L. argyrognomon* do not lay eggs on *V. cracca* in the field. The possibility that *L. argyrognomon* uses CHA-*I* as a host plant is discussed.

Key words Chinese-grown *Indigofera* sp., *Indigofera pseudo-tinctoria*, *Lycaeides argyrognomon*, oviposition preference, *Vicia cracca*.

Introduction

Lycaeides argyrognomon (Bergsträsser) is a grassland lycaenid butterfly designated as Endangered (EN) by the Ministry of the Environment (2012). This butterfly is distributed in Japan, the Korean Peninsula, Northeastern China, Europe and North America (Shirozu, 2006). In Japan, the larvae of this butterfly feed only on *Indigofera pseudo-tinctoria* (JPN-*I*) (Fukuda *et al.*, 1984). In contrast, many leguminous plants have been reported as host plants of *L. argyrognomon* larvae in Europe and North America (Takahashi, 2007).

Koda and Nakamura (2011) confirmed that *L. argyrognomon* larvae given Chinese-grown *Indigofera* sp.(CHA-*I*) were able to grow well to adulthood. This plant was utilised in revegetational technology used on roadside slopes in Japan through 1990 (Aiba and Natsume, 1995; Yoshida, 2008). Additionally, it has been reported that the survival rate of larvae of this species reared on *Vicia cracca* is 45.5% (Koda *et al.*, 2012). These studies suggest that Japanese larvae of the species may have the potential to feed on leguminous plants other than JPN-*I*.

It has been reported that in Japan, larvae of *L. argyrognomon* feed only on JPN-*I* because adult females lay eggs only on JPN-*I* (Fukuda *et al.*, 1984). The purpose of the present study is to examine whether adult females of *L. argyrognomon* may lay eggs on plants other than JPN-*I*.

Materials and methods

Insects

Adult females of *L. argyrognomon* were collected from several spots along the Otagiri River in Komagane City, Nagano Prefecture, Japan, on July 29, August 6, 2011.

Plant species

We used three leguminous species as test plants (Fig.1). JPN-*I* was collected at the campus of the Faculty of Agriculture, Shinshu University and CHA-*I* was collected from a roadside slope in Minowa City, Nagano Prefecture, Japan, where it has been planted artificially for slope revegetation. *V. cracca* was collected in Ina City, Nagano Prefecture, Japan.

Oviposition test

All experiments were carried out at room temperature with a photoperiod of 16L: 8D in the laboratory of the Faculty of Agriculture of Shinshu University. We used the Richard method to make female butterflies lay eggs

*Corresponding author, E-mail: kkeiko12@shinshu-u.ac.jp

compulsorily. Each female caught in the field was placed within the oviposition equipment containing twigs of test plants (Fig. 2), and then allowed to oviposit from 10:00 to 16:00. To avoid too hot conditions the filament lamp was turned on and off at 1hr intervals. During this time, temperature in the equipment was about 27-30˚C. Eggs laid down were daily collected, and then the number of eggs per day was calculated. After this treatment, each female was put in a cylindrical cage (220 mm in diameter, 150 mm high) made of polyester cloth and then given sugar solution as food.

Experiment 1

This experiment was carried out on August 1, 3 and 5 in 2011. A total of nine sets of oviposition equipment (3 of JPN-*I*, 3 of CHA-*I* and 3 of *V. cracca*) were prepared. Nine females were divided into three groups (A, B and C) composed of three individuals each and then were put into the equipment with the rotation sequence shown in Table 1. Two twigs 25 and 30 cm in height were used as oviposition plants in the equipment.

Fig. 1. Three plants used in this study. A: *Indigofera pseudo-tinctoria* (JPN-*I*); B: *Indigofera* sp. (CHA-*I*); C: *Vicia cracca*.

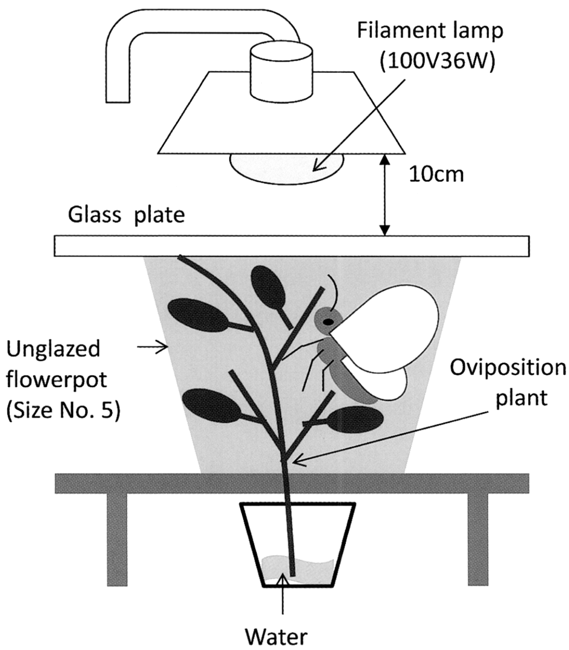

Fig. 2. A sketch drawing of the oviposition equipment following the Richard method.

Table 1. The number of eggs laid on three plants in Experiment 1.

Rotation group	Invididual number	*Indigofera pseudo-tinctoria* (JPN-*I*)		*Indigofera* sp. (CHA-*I*)		*Vicia cracca*		Total
		Date	No. of eggs	Date	No. of eggs	Date	No. of eggs	
A	1	8/1	19	8/3	0	8/5	0	19
	2	8/1	48	8/3	13	8/5	0	61
	3	8/1	100	8/3	45	8/5	0	145
B	4	8/5	37	8/1	0	8/3	0	37
	5	8/5	11	8/1	0	8/3	0	11
	6	8/5	28	8/1	13	8/3	0	41
C	7	8/3	63	8/5	12	8/1	0	75
	8	8/3	8	8/5	7	8/1	0	15
	9	8/3	58	8/5	16	8/1	0	74
Total		—	372	—	106	—	0	478
%		—	77.8%	—	22.2%	—	0%	100%

Experiment 2

This experiment was performed on August 8, 9, 11 and 12 in 2011. Each of five females was put into the oviposition equipment with one JPN-*I* twig and one CHA-*I* twig 30 cm in height.

Statistical analysis

For statistical analyses, we employed the Friedman test using the data set of all individuals in Experiment 1, and Wilcoxon rank sum test in Experiment 2.

Results

Experiment 1

The numbers of eggs which were laid on the three leguminous plants (JPN-*I*, CHA-*I* and *V. cracca*) are shown in Table 1. The number of eggs laid by one female ranged from 145 to 11. All females laid eggs on JPN-*I* while two-thirds of females (66.7%) laid on CHA-*I*. By contrast, no females laid eggs on *V. cracca*. There were significant differences in the numbers of eggs laid on the three plants ($p = 2.13 \times 10^{-4}$; Friedman test for the data set of total females).

Experiment 2

The numbers of eggs laid on JPN-*I* and CHA-*I* are shown in Table 2. All females laid eggs on both JPN-*I* and CHA-*I*. The number of eggs laid was greater on JPN-*I* than on CHA-*I*. Numbers of eggs ranged from 169 to 71. For egg numbers, a significant difference was found between JPN-*I* and CHA-*I* ($p < 0.01$; Wilcoxon rank sum test for the data set of 5 females).

Discussion

There have been some reports of *L. argyrognomon* feeding on plants other than JPN-*I* in Japan (Entomological Society of Shinshu, 1976; Nihira, 2004; Yago, 2007). Additionally, it has been reported that the survival rates of *L. argyrognomon* larvae reared on CHA-*I* and *V. cracca* are 69.2% and 45.5%, respectively (Koda and Nakamura, 2011; Koda *et al.*, 2012). Based on these studies, it can be said that *L. argyrognomon* larvae of Japan are able to feed on some leguminous plants other than JPN-*I* and grow to adulthood. In the present study, we found that adult females of *L. argyrognomon* did not lay eggs on *V. cracca,* even though larvae are able to feed on it (Table 2). Thus, we believe that *L. argyrognomon* larvae do not feed on *V. cracca* in the field because females do not lay eggs on it. In this study, we used field-caught females, but oviposition experiences may influence oviposition preference (Hirota and Kato, 2004). For this reason, the optimum course is to use females which have not yet laid. We need to establish a technique for cage pairing of *L. argyrognomon* adults reared in the laboratory.

Watanabe and Miyashita (2006) report that *Lycaeides subsolanus* larvae were found to feed on *Vicia pseudo-orobus*, which was not known as a host plant of this butterfly at the time. They suggested that *L. subsolanus* larvae might feed on *V. pseudo-orobus* because it is distributed together with *Vicia unijuga*, which is the primary host plant of this butterfly. Tashita *et al.*, (2013) found eggs and larvae of *L. argyrognomon* on *Melilotus officinalis alba*, which had not been known as a host plant. Thus, we believe that, as in these cases, *L. argyrognomon* larvae may feed on *V. cracca*, if it is distributed together with JPN-*I*.

Table 2. The number of eggs laid on *Indigofera pseudo-tinctoria* (JPN-*I*) and *Indigofera* sp. (CHA-*I*) in Experiment 2.

Invididual number	8/8		8/9		8/11		8/12		Total	
	JPN-*I*	CHA-*I*	JPN-*I*	CHA-*I*	JPN-*I*	CHA-*I*	JPN-*I*	CHA-*I*	JPN-*I*	CHA-*I*
10	13	6	38	2	18	2	10	0	79	10
11*	64	1	6	0	–	–	–	–	70	1
12	25	3	28	2	10	1	3	0	66	6
13	33	7	30	3	7	13	26	0	96	23
14	36	10	44	13	52	0	13	1	145	24
Total	171	27	146	20	87	16	52	1	456	64
%	86.4%	13.6%	88.0%	12.0%	84.5%	15.5%	98.1%	1.9%	87.7%	12.3%

*: Individual No. 11 died on August 11.

Larvae of a pierid butterfly *Eurema laeta* were believed for a long time to feed only on *Chamaecrista nomame* in Japan (Shirozu, 2006). However, it was recently reported that populations of *E. laeta* in Shizuoka, Aichi and Gifu Prefectures have increased because of host shift from *C. nomame* to *Chamaecrista nictitans*, which is a naturalized plant (Ueyama, 2009). In recent times, CHA-*I* has been imported from China for use in slope revegetation in Japan. There is the obvious probability that *L. argyrognomon* may change its host plant from JPN-*I* to CHA-*I*, as in the case of *E. laeta*.

Koda and Nakamura (2011) pointed out two deciding factors in determining whether *L. argyrognomon* can use CHA-*I* as a host plant: 1) Does the adult butterfly lay eggs on CHA-*I*? and 2) Does the adult butterfly move around its habitat and find CHA-*I*? In the present study, we found that 66.7% of *L. argyrognomon* females laid eggs on CHA-*I* in Experiment 1 (Table 2) and all females laid eggs on CHA-*I* in Experiment 2 (Table 3). Koda and Nakamura (2011) report that there were no significant differences in the survival rate between eggs laid on JPN-*I* and CHA-*I* and the pupal weight of individuals reared on CHA-*I* was heavier than that of those on JPN-*I*. Based on these results, the possibility that *L. argyrognomon* may use CHA-*I* as a host plant is considered to be high, where CHA-*I* is distributed together with JPN-*I*. Further research is necessary to investigate whether *L. argyrognomon* is found in areas where CHA-*I* is growing.

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摘　要

3種の植物（在来コマツナギ，中国産コマツナギ，クサフジ）に対するミヤマシジミのメス成虫の産卵選択（尾崎絵理・江田慧子・中村寛志）

在来コマツナギ，中国産コマツナギおよびクサフジに対するミヤマシジミのメス成虫の産卵選択を調べるために，2011年にリシャール法を用いた2つの実験を信州大学農学部昆虫生態学研究室で行った．3種の植物を順番に用いて産卵させた実験では，在来コマツナギへは全ての個体が産卵したが，中国産コマツナギには実験に供した9個体中6個体（66.7%）が産卵した．またクサフジにはどのメスも産卵しなかった．在来コマツナギへの産卵数は372卵(77.8%)であったのに対して，中国産コマツナギへの産卵数は106卵（22.2%）であった．在来コマツナギと中国産コマツナギを一緒に用いた実験では，すべてのメス（5個体）が中国産コマツナギに産卵した．この実験での在来コマツナギへの産卵数は456卵（87.7%）であったのに対して，中国産コマツナギへの産卵数は64卵（12.3%）であった．この結果より，野外ではミヤマシジミ幼虫がクサフジを食べないのは，メス成虫がクサフジに産卵しないからであると考えられた．またミヤマシジミが中国産コマツナギを食草とする可能性について考察した．

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